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(54) **Title:** FRACTIONATION USING DIELECTROPHORESIS AND FIELD FLOW FRACTIONATION

(57) **Abstract**

The present disclosure is directed to a novel apparatus and novel methods for the separation, characterization, and manipulation of matter. The invention combines the use of frequency-dependent dielectric and conductive properties of particulate matter and solubilized matter with the properties of the suspending and transporting medium to discriminate and separate such matter. The apparatus includes a chamber having at least one electrode element and at least one inlet and one output port into which matter are introduced and removed from the chamber. Matter carried through the chamber in a fluid stream is then displaced within the fluid by a dielectrophoretic (DEP) force caused by the energized electrode. Following displacement within the fluid, matter travels through the chamber at velocities according to the velocity profile of the chamber. After the matter has transitted through the chamber, it exits at the opposite end of the chamber at a characteristic position.

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DESCRIPTION

FRACTIONATION USING DIELECTROPHORESIS AND FIELD FLOW FRACTIONATION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of molecular separation and particle discrimination. More particularly, it concerns the fractionation of particulate matter utilizing a combination of electrical, hydrodynamic or gravitational forces.

2. Description of the Related Art

The ability to identify, characterize and purify cell subpopulations is fundamental to numerous biological and medical applications, often forming the starting point for research protocols and the basis for current and emerging clinical protocols. Cell separation has numerous applications in medicine, biotechnology, and research in environmental settings. For example, cell separation can make possible life-saving procedures such as autologous bone marrow transplantation for the remediation of advanced cancers where the removal of cancer-causing metastatic cells from a patient's marrow is necessitated (Fischer, 1993). In other applications, such as the study of signaling between blood cells (Stout, 1993), (Cantrell *et al.*, 1992), highly purified cell subpopulations permit studies that would otherwise be impossible. Current approaches to cell sorting most frequently exploit differences in cell density (Boyum, 1974), specific immunologic targets (Smeland *et al.*, 1992), or receptor-ligand interactions (Chess and Schlossman, 1976) to isolate particular cells.

These techniques are often inadequate and sorting devices capable of identifying and selectively manipulating cells through novel physical properties are therefore desirable. The application of the principles of AC electrokinetics has been used for the dielectric characterization of mammalian cells through the method of electrorotation (ROT) (Arnold and Zimmermann, 1982; Fuhr, 1985; Hölzel and Lamprecht, 1992; Wang *et al.*, 1994) and for cell discrimination and sorting (Hagedorn *et al.*, 1992; Huang *et al.*, 1993; Gascoyne *et al.*, 1992; Gascoyne *et al.*, 1994; Huang *et al.*, 1992). In these techniques, cells become electrically polarized when they are subjected to an AC electric field. If that field is inhomogeneous, then the cells experience a lateral dielectrophoretic (DEP) force, the frequency response of which is a function of their intrinsic electrical properties (Gascoyne *et al.*, 1992). In turn, these properties depend strongly on cell composition and organization, features that reflect cell morphology and phenotype. Cells differing in their electrical polarizabilities can thus experience differential forces in the inhomogeneous electric field (Becker *et al.*, 1994; Becker *et al.*, 1995). Analysis of the dielectrophoretic motion of mammalian cells as a function of applied frequency permits cell membrane biophysical parameters, such as capacitance and surface conductance, to be probed. Because DEP effectively maps biophysical properties into a translational force whose direction and magnitude reflects cellular properties, some degree of separation occurs between particles of different characteristics. While DEP has been used on a microscopic scale to separate bacteria from erythrocytes (Markx *et al.*, 1994), viable from nonviable yeast cells (Wang *et al.*, 1993), and erythroleukemia cells from erythrocytes (Huang *et al.*, 1992), the differences in the electrical polarizabilities of the cell types in those various mixtures were greater than those to be expected in many typical cell sorting applications.

Field flow fractionation (FFF) has also been generally employed for separation of matter, utilizing particle density, size, volume, diffusivity, thickness, and surface charge as parameters (Giddings, 1993). The technique can be used to separate many

different types of matter, from a size of about 1 nm to more than about 100 micrometers which may include, for example, biological and non-biological matter. Separation according to field flow fractionation occurs by differential retention in a stream of liquid flowing through a thin channel. The FFF technique combines elements of chromatography, electrophoresis, and ultracentrifugation, and generally FFF requires the presence of a field or gradient to develop a differential flow. This differential flow creates a flow profile which may be, for example, linear or parabolic. A field is then applied at right angles to the flow and serves to drive the matter into different displacements within the flow profile which travel at differing velocities. Fields may be based on sedimentation, crossflow, temperature gradient, centrifugal forces, and the like. The technique suffers, however, from producing insufficiently pure cell populations, being too slow, or being too limited in the spectrum of target cells or other matter.

Thus, there exists a need in the art for highly discriminate separation of particulate matter, especially biological matter, that operates without physically modifying the structure of the matter to be separated. Moreover, such an approach should allow for the sensitive manipulation of such particles, which may include characterization and purification of desired matter from extraneous or undesired matter.

SUMMARY OF THE INVENTION

The present invention seeks to overcome these drawbacks inherent in the prior art by combining the use of frequency-dependent dielectric and conductive properties of particles with the properties of the suspending and transporting medium. As used herein, the term "matter" is intended to include particulate matter, solubilized matter, or any combination thereof. The invention provides a novel apparatus and novel methods by which different particulate matter and solubilized matter may be identified and selectively manipulated. These particles may also be collected by changing the DEP

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